Polymorphisms in glutathione-S-transferase genes (*GST-M1*, *GST-T1* and *GST-P1*) and susceptibility to prostate cancer among male smokers of the ATBC cancer prevention study

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Glutathione-*S*-transferase (GST) genes encode a family of detoxification enzymes that offer protection against endogenous and exogenous sources of reactive oxygen species (ROS). Germline variations in GST genes may alter the catalytic efficiency of GST isoenzymes leading to a potential increase in susceptibility to the genotoxic effects of ROS and electrophilic substances. A nested case–control study design was used to examine the association between the polymorphic GST genes and prostate cancer risk among Finnish male smokers of the ATBC Cancer Prevention Study. A case–case analysis was used to determine the association between these genetic polymorphisms and prostate cancer progression. Germline DNA was obtained from 206 prostate cancer cases and 194 controls frequency matched on age, intervention group and study clinic. Cases and controls were genotyped for three GST genes using MALDI-TOF mass spectrometry or multiplex polymerase chain reaction (PCR). Relative to the wild-type genotype, we observed a 36% reduction in prostate cancer risk associated with the *GST-M1*-null genotype (odds ratio (OR) 0.64, 95% confidence interval (CI) 0.43, 0.95). Unlike *GST-M1*, *GST-T1*-null (OR 0.74, 95% CI 0.42, 1.33) and *GST-P1*B* (OR 1.10, 95% CI 0.72, 1.69) were not strongly associated with prostate cancer risk. We did not observe any significant associations between the selected polymorphic GST genes and tumour grade or stage. In conclusion, we did not observe a direct association between polymorphic *GST-T1* or *GST-P1* and prostate cancer risk. Our observation of a relatively strong inverse association between the *GST-M1*-null genotype and prostate cancer risk needs to be confirmed in larger association studies. *European Journal of Cancer Prevention* 12:317–320 © 2003 Lippincott Williams & Wilkins.

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Introduction

Glutathione-*S*-transferases (GSTs) are a class of detoxification or antioxidant enzymes that catalyse the conjugation of reduced glutathione to electrophilic compounds, including endogenous or exogenous sources of reactive oxygen species (ROS) and environmental carcinogenic compounds (e.g., benzo[a]pyrenediol epoxides) (Hayes and Pulford, 1995). The following allelic variants of selected polymorphic GST genes have been previously reported: homozygous deletion alleles (i.e. *GST-M1*0* and *GST-T1*0*) for both polymorphic *GST-M1* and *GST-T1*; and *GST-P1*B* allele, which has an adenosine-313/guanosine nucleotide substitution in exon 5, resulting in an Ile105 to Val amino acid substitution within the active site of the enzyme (Hayes and Pulford, 1995). The allele frequencies of *GST-M1*0*, *GST-T1*0* and *GST-P1*B*, range from 40 to 60%, 15–30% and 32–52% within Caucasian populations, respectively (Seidegard *et al.*, 1988; Harries *et al.*, 1997; Autrup *et al.*, 1999; El Masri *et al.*, 1999; Rebbeck *et al.*, 1999; Woodson *et al.*, 1999; Shepard *et al.*, 2000; Steinhoff *et al.*, 2000; Kote-Jarai *et al.*, 2001; Krajinovic *et al.*, 2001). We hypothesize that deletion polymorphisms in *GST-M1* and *GST-T1* may modulate the conjugation or detoxification capacity of respective GST isoenzymes to detoxify environmental carcinogens, resulting in increased susceptibility to the detrimental effects of endogenous and exogenous ROS and subsequent increased prostate cancer (PCA) risk. Moreover, the *GST-P1*B* variant allele, linked to increased conjugation of biologically active electrophiles (i.e. BPDE) to glutathione (Hu *et al.*, 1997a,b; Sundberg *et al.*, 1998), may be associated with a reduced PCA risk, presumably due to a reduction in exogenously generated ROS.

Genetic variations in polymorphic GST genes have been implicated in the aetiology of numerous cancers, including PCA (Coughlin and Hall, 2002). Recently, Steinhoff *et al.* observed a significant 2.3-fold increase in prostate cancer risk associated with the *GST-T1* deletion polymorph-

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ism (Steinhoff *et al.*, 2000). The *GST-M1*-null genotype has been associated with an increased risk of lung, bladder, breast and colon cancers (Strange *et al.*, 1991; Bell *et al.*, 1992). In terms of polymorphic *GST-P1*, Gsur *et al.* (2001) demonstrated a significant reduction (35–77%) in PCA risk among individuals who were homo-/heterozygous variant for the *GST-P1*B* variant allele.

The major goal of this study is to examine the association between germline variations in polymorphic GST genes (*GST-T1*, *GST-M1* and *GST-P1*) and prostate cancer risk, histological grade and clinical stage among Finnish male participants of the Alpha Tocopherol Beta Carotene (ATBC) cancer prevention study. In addition, we explored whether the inheritance of multiple variant GST genes was associated with increased PCA risk when compared with non-variants.

Materials and methods

Cases and controls were identified among participants of the ATBC cancer prevention trial (ATBC Cancer Prevention Study Group; Albanes *et al.*, 1995, 1996). The ATBC Study was approved by the institutional review boards of the National Cancer Institute (USA) and the National Public Health Institute of Finland. Upon entry, all study participants provided written informed consent.

A nested case–control sample set was constructed based on the availability of participants ($n = 20\,305$) who had a whole blood sample collected at the end of the study as detailed previously (Woodson *et al.*, 2003). Among these, 206 incident cases of primary PCA (ICD-185) were diagnosed between 1985 and 1994. Study oncologists and pathologists centrally reviewed the medical records and histopathologic/cytologic specimens of cases, respectively. The percentage of cases diagnosed with localized disease (stage 0–II), regional (stage III) and remote disease (stage IV) were 64%, 12% and 24%, respectively. Approximately 41%, 42% and 17% of the cases had well (grade 1: roughly equivalent to Gleason grade 1–4), moderately (grade 2: Gleason grade 5–7) and poorly differentiated tumours (grade 3: Gleason grade 8–10). Controls ($n = 194$) were frequency-matched to cases ($n = 206$) on age (5 years), intervention group and study clinic.

Genomic DNA was isolated from available whole blood samples, as detailed previously (Woodson *et al.*, 2003). Prior to *GST-P1* genotyping, the isolated DNA was subjected to a polymerase chain reaction (PCR) in order to amplify a small region containing the single nucleotide substitution within *GST-P1* (i.e. *GST-P1* adenosine-313/guanosine). The PCR reaction required for GST-P1 genotyping required PCR primers, namely a sense primer 5′-acg ttg gat caa ccc tgg tgc aga tgc tc-3′, antisense primer 5′-acg ttg gat tgg tgg aca tgg tga atg ac-3′, and extension primer 5′-gga cct ccg ctg caa ata c-3′. Following PCR amplification, the PCR products were genotyped by Sequenom's mass array technology, which combines MassExtend primer extension, SpectroCHIP arrays and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Sequenom, San Diego, CA, USA). Procedures for the PCR reaction, themocycler program, primer extension reaction, primer extension product purification and mass spectral analysis has been detailed previously (Woodson *et al.*, 2003, in press).

We determined the presence or absence of the *GST-M1* and *GST-T1* genotypes using a modified version of a multiplex PCR detailed elsewhere (Bell *et al.*, 1992, 1993). Isolated DNA (50 ng) was added to a PCR mixture consisting of the following: 20 pmol of each primer, 2.0 mmol/l of each dNTP, 2.5 U MBI Taq polymerase, 1 × PCR buffer (67 mmol/l βTris–HCl at pH 8.8, 50 mmol/l -ME, 16.6 mmol/l $(NH_4)_2SO_4$, 6.8 mol/l EDTA and 80 g BSA), and 3.3 mmol/l $MgCl_2$ in a final volume of 25 l. In order to PCR amplify short DNA fragments of *GST-M1* and *GST-T1*, the following primers were used: *GST-M1* primers (sense 5′-gaa ctc cct gaa aag cta aag c-3′ and antisense 5′-gtt ggg ctc aaa tat acg gtg g-3′); *GST-T1* primers (sense 5′-ttc ctt act ggt cct cac atc tc-3′ and antisense 5′-tca ccg gat cat ggc cag ca-3′); and beta-globin primers (sense 5′-caa ctt cat cca cgt tca cc-3′ and antisense 5′-gaa gag cca agg aca ggt ac-3′). The Perkin Elmer Cetus thermal cycler program involved 35 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min.

The chi-squared test was used to test the hypothesis that the distribution of variant genotypes was similar between cases and controls. The associations between polymorphic GST genes and PCA risk or clinicopathologic characteristics (e.g. tumour grade or stage) are expressed as odds ratios and corresponding 95% confidence intervals and were estimated using unconditional logistic regression analysis. All reported odds ratios and corresponding 95% confidence intervals (OR, 95% CI) for polymorphisms in *GST-T1*, *GST-M1* and *GST-P1* were relative to the following wild-type alleles: *GST-T1*1* (presence of at least one undeleted *GST-T1* allele), *GST-M1*1*, *GST-P1*A/*A*. Covariates were included in the regression model if they changed the OR by > 20% or significantly altered the likelihood ratio statistic. Since matching factors, demographic characteristics, lifestyle factors, serum antioxidant levels and occupational exposure did not significantly change risk estimates, univariate estimates are reported.

Results

The frequency of the *GST-T1*-null and *GST-P1*B* variant alleles did not differ by case–control status; however, the frequency of the *GST-M1* deletion alleles was lower among cases when compared with controls (Table 1). There were no significant differences in allelic frequen-

Table 1. Association between polymorphic GST genes and prostate cancer risk and disease progression

	Cases	Controls	OR (95% CI)[f]
GST-M1			
(*1/*1 + *1/*0)[a]	116 (58)	88 (47)	Reference
*0/*0[b]	84 (42)	100 (53)	0.64 (0.43, 0.95)
GST-T1			
(*1/*1 + 1/0)	178 (88)	160 (85)	Reference
0/*0[b]	24 (12)	29 (15)	0.74 (0.42, 1.33)
GST-P1			
A/A	92 (54)	95 (57)	Reference
(B/B + *B/A)[c]	78 (46)	73 (43)	1.10 (0.72, 1.69)
	Grade 1–2	Grade 3[d]	OR (95% CI)[f]
GST-M1			
(1/1 + 1/*0)	83 (59)	15 (54)	Reference
0/0	59 (42)	13 (46)	1.22 (0.54, 2.75)
GST-T1			
(1/1 + *1/0)	125 (87)	24 (89)	Reference
0/0	19 (13)	3 (11)	0.82 (0.23, 3.00)
GST-P1			
A/*A	62 (52)	12 (57)	Reference
(B/B + B/A)	58 (48)	3 (11)	0.8 (0.32, 2.04)
	Stage 0–II	Stage III–IV[e]	OR (95% CI)[f]
GST-M1			
(*1/1 + 1/0)	74 (57)	42 (60)	Reference
0/*0	56 (43)	28 (40)	0.88 (0.48, 1.59)
GST-T1			
(*1/*1 + *1/*0)	117 (90)	61 (85)	Reference
*0/*0	13 (10)	11 (15)	1.62 (0.69, 3.84)
GST-P1			
*A/*A	54 (49)	38 (63)	Reference
(*B/*B + *B/*A)	56 (51)	22 (37)	0.56 (0.29, 1.06)

[a]Men with at least one *GST-T1* or *GST-M1* undeleted allele.
[b]Men with two *GST-T1* or *GST-M1* deleted alleles.
[c]Men with heterozygous or homozygous *GST-P1*B* variant alleles.
[d]High tumour grade (poorly differentiated) versus low grade (low to moderately differentiated).
[e]Advanced (regional/remote PCA tumours) versus non-advanced (localized PCA tumours) differentiated PCA tumour stage.
[f]Odds ratio of prostate cancer risk comparing individuals with variant genotypes to those with wild-type genotypes, using an unconditional logistic regression analysis model. Risk estimates adjusted for potential confounders (i.e. matching factors age, body mass index, smoking status, micronutrient intakes and occupational status) were not significantly different relative to unadjusted risk estimates. Thus, unadjusted risk estimates are reported.

cies of *GST-M1*0*, *GST-T1*0* and *GST-P1*B* alleles between cases with poorly differentiated (Gleason grade 8–10) or regional/remote (TNM tumour stage $\geqslant 3$) tumours when compared with those diagnosed with well/moderately differentiated (Gleason grade $\leqslant 7$) or localized tumours (TNM stage $\leqslant 2$), respectively. In an exploratory analysis, the inheritance of one or more variant GST genes was not associated with an elevated risk for prostate cancer susceptibility when compared with non-variants (χ^2 P trend = 0.61).

Discussion

Our data indicate that germline variations within polymorphic *GST-T1* and *GST-P1* genes may not play a major role in PCA risk. Our observation of a modest inverse association between the *GST-M1* deletion genotype and PCA risk was not in accordance with other investigations that reported null findings on this relationship (Kelada *et al.*, 2000). A plausible post hoc explanation for this inverse relationship may be related to the role GSTs play in the intracellular transport of steroids and metabolic activation of androgens (Listowsky *et al.*, 1988; Petersson and Mannervik, 2001). Perhaps the loss of *GST-M1* activity may be related to either decreased metabolic conversion of androst-5-ene-3,17-dione to an immediate precursor of testosterone or reduced testosterone protein binding, resulting in reduced bioavailability of activated androgens necessary for prostate tumorigenesis. Although our study had limited power to detect more modest associations between these polymorphisms and prostate cancer risk, we can conclude that none of the selected polymorphic genes were strongly associated with risk or disease grade and stage. However, we had sufficient statistical power to rule out positive associations of the magnitude previously reported in the literature between polymorphic GSTs (i.e. homozygous deletion *GST-T1* and *GST-P1*B* alleles) and PCA (Harries *et al.*, 1997; Steinhoff *et al.*, 2000).

It is possible that germline genetic susceptibilities in a single GST gene may not have a significant impact on PCA risk, particularly if other GST genes compensate for its activity. This compensation, however, may only offer partial protection against biologically active electrophiles and reactive oxygen species, since GSTs have variable affinities toward different substrates (Hu *et al.*, 1997a,b;

Landi, 2000). Alternatively, individuals may have to inherit a multitude of putative high-risk alleles within polymorphic GST genes in order to experience a decrease in the capacity of GST isoenzymes to detoxify environmental carcinogens leading to elevated PCA risk. In an exploratory analysis, we tested this hypothesis, but did not observe any significant gene–gene interactions among the three polymorphic genes in this study.

In conclusion, our data indicate that germline variations in polymorphic GST genes may not play a major role in PCA risk. Our observation of a modest inverse association between the *GST-M1* deletion genotype and PCA risk needs to be confirmed in other studies, particularly those that are statistically powered to investigate whether serum hormone levels may modify the association between polymorphic *GST-M1* and PCA risk.

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